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Developmental Differences in White Matter Architecture Between Boys and Girls

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Abstract: Previous studies have found developmental differences between males and females in brain structure. During childhood and adolescence, relative white matter volume increases faster in boys than in girls. Sex differences in the development of white matter microstructure were investigated in a cohort of normal children ages 5–18 in a cross-sectional diffusion tensor imaging (DTI) study. Greater fractional anisotropy (FA) in boys was shown in associative white matter regions (including the frontal lobes), while greater FA in girls was shown in the splenium of the corpus callosum. Greater mean diffusivity (MD) in boys was shown in the corticospinal tract and in frontal white matter in the right hemisphere; greater MD in girls was shown in occipito-parietal regions and the most superior aspect of the corticospinal tract in the right hemisphere. Significant sex–age interactions on FA and MD were also shown. Girls displayed a greater rate of fiber density increase with age when compared with boys in associative regions (reflected in MD values). However, girls displayed a trend toward increased organization with age (reflected in FA values) only in the right hemisphere, while boys displayed this trend only in the left hemisphere. These results indicate differing developmental trajectories in white matter for boys and girls and the importance of taking sex into account in developmental DTI studies. The results also may have implications for the study of the relationship of brain architecture with intelligence. *Hum Brain Mapp 29:696–710, 2008.* © 2007 Wiley-Liss, Inc.

Key words: age factors; brain growth and development; diffusion tensor imaging; sex differences

INTRODUCTION

Sex differences in brain structure and pathology have been shown for various pathologies such as alcoholism [Pfefferbaum et al., 2001] and schizophrenia [Highley et al., 2003]. Since the advent of magnetic resonance imaging (MRI) of the brain, researchers have been able to examine sex differences in brain structure and function in more detail than was possible previously, when researchers would have to rely mainly on postmortem histologies. A greater knowledge of sexual dimorphism in the brains of normal healthy individuals and its developmental implications should be useful for a better understanding of the neuroanatomical underpinnings of various pathologies.

Across all age ranges, many studies have shown that males have greater total cerebral volume, as well as total gray and total white matter volumes compared to females [Allen et al., 2003; Blatter et al., 1995; Caviness et al., 1996; De Bellis et al., 2001; Filipek et al., 1994; Nopoulos et al., 2000; Reiss et al., 1996]. When gray and white matter volumes are normalized to total cerebral volume, however, adult men possess a greater proportion of white matter compared to adult women, who possess a correspondingly greater proportion of gray matter relative to total cerebral

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volume [Allen et al., 2003; Gur et al., 1999]; this differentiation has been found to be regionally specific [Luders et al., 2005]. Absolute brain size differences between adult men and women are mostly attributable to differences in white matter volume [Passe et al., 1997], as are regional differences in gray/white matter volume ratio [Allen et al., 2003].

Using more advanced MRI techniques, sexual dimorphism in adults has been shown in a variety of additional domains. For instance, a recent diffusion tensor imaging (DTI) study found greater diffusion anisotropy in the left frontal lobe of adult women as compared to men [Szeszko et al., 2003]. Using functional MRI, sex differences in the neural correlates of language function have been shown [Shaywitz et al., 1995]. Structural and neuroanatomical differences between adult men and women would likely reflect differences in the correlation of brain architecture with cognitive function. Accordingly, sex differences in the neuroanatomical correlates of intelligence have been noted [Andreasen et al., 1993], with women displaying a greater dependence on white matter volume and men a greater dependence on gray matter volume [Gur et al., 1999], a finding which has been recently replicated and found to be regionally specific [Haier et al., 2005]. Magnetic resonance spectroscopy (MRS) has been used to measure *N*-acetyl-aspartate (NAA) concentrations, and NAA levels in frontal and parietal regions have been found to correlate with intelligence in women but not in men [Jung et al., 2005; Pfleiderer et al., 2004].

Of particular interest are developmental implications of sexual dimorphism in the brain. While most brain structures scale uniformly to the adult brain size by the age of 7 in both sexes, the subcortical gray matter volume is relatively larger in boys, and the volume of central white matter is smaller in boys, indicating a regression in relative subcortical gray matter in boys, and a relatively larger increase in central white matter volume occurring between the age of 7 and adulthood [Caviness et al., 1996]. Boys ages 5–18 also display greater variance in white matter volume compared to girls [Reiss et al., 1996]. Normalizing for whole brain size, a significant sex–age interaction has been demonstrated, with boys displaying greater rates of increase with age of normalized white matter volume, and greater rates of decrease with age of normalized gray matter volume, relative to girls between the ages of 7–17 [De Bellis et al., 2001]. Absolute white matter volume in the left inferior frontal gyrus was shown to increase in boys but not in girls [Blanton et al., 2004]; relative gray matter volume (normalized for total intracranial volume) in the left inferior frontal gyrus nevertheless remained larger in boys.

A sexually dimorphic development of brain structure may cause developmental differences between boys and girls in the relationship of brain structure to cognitive function. Differences in functional activation between boys and girls for various elements of language processing have been shown [Plante et al., 2006]. The relationship between functional connectivity utilized for language processing and intelligence also appears to differ between boys and girls [Schmithorst and Holland, 2006, 2007]. More intelligent boys develop a more modular functional architecture with age, while more intelligent girls develop a more connected functional architecture with age [Schmithorst and Holland, 2006]; girls develop a greater reliance on interhemispheric connectivity for intelligence with age, while boys develop a greater reliance on specific connectivity with the left inferior frontal gyrus (Broca's area) [Schmithorst and Holland, 2007].

These results, showing possible differences in brain maturational processes between boys and girls, lead us to investigate these differences further using DTI. Previously, it was found that diffusion anisotropy increases with age in regionally specific white matter regions including the internal capsule and arcuate fasciculus, while mean diffusivity decreases with age throughout the white matter [Schmithorst et al., 2002]. A sexual dimorphism in this maturational process is hypothesized, the specifics of which are outlined below.

We predict a greater degree of anisotropy overall in males. Males possess a greater number of neurons and fewer neuronal processes [de Courten-Myers, 1999], with greater absolute and relative white matter volume available for the inter-neuronal connections [Allen et al., 2003; Filipek et al., 1994; Gur et al., 1999]. Thus, one might expect males to possess fewer, but thicker, more organized, and possibly more myelinated fibers, with females possessing more crossing fiber tracts. In adult men, higher anisotropy has been observed in the corpus callosum, hypothesized to be associated with increased myelination [Westerhausen et al., 2003, 2004], despite greater fiber density in females [Highley et al., 1999]. This phenomenon, however, will likely be regionally specific, depending on the specific neuronal connections involved.

We also hypothesize a developmental effect related to gray matter "pruning" [Casey et al., 2000; Courchesne et al., 2000; Huttenlocher and Dabholkar, 1997; Huttenlocher and de Courten, 1987]. Elimination of inefficient or unnecessary neuronal connections, as well as the maturation of other neuronal connections, especially in the frontal lobe [Schlaggar et al., 2002] and corticospinal tract [Schmithorst et al., 2002], results in increased fiber organization. Therefore, we would expect to find specific regions exhibiting sex–age interaction effects on diffusion anisotropy, reflecting developmental differences in brain organization between girls and boys.

We predict that boys will exhibit greater mean diffusivity throughout the brain, reflecting fewer neuronal processes but a larger volume of white matter. In addition, we also expect that mean diffusivity will exhibit a sex–age interaction effect, reflecting the sex–age interaction previously seen for the gray/white ratio [De Bellis et al., 2001]. These effects may be regionally specific. In some regions such as the central white matter and the relative white matter volume increases in girls and approaches that of boys [Caviness et al., 1996]; in other areas, such as the left

inferior frontal gyrus, the relative gray matter remains larger in boys [Blanton et al., 2004].

MATERIALS AND METHODS

Subjects were recruited from an fMRI imaging study of normal language development, to which a DTI acquisition was added. Institutional Review Board approval was obtained for the study and informed consent of parents (with assent of children over the age of 8) was obtained for all subjects. Subjects were recruited via advertisements broadcast on local television stations and newspapers, and posted in primary care clinics within the hospital and in the metropolitan area. Healthy siblings of patients at our hospital were also invited to participate. All potential participants were pre-screened by questionnaire and structured telephone interview for any conditions (such as the presence of orthodontic braces) which would prevent a high-quality MRI scan from being acquired.

All children underwent a brief neurological examination administered by a pediatric neurologist and subjects who did not test within the normal range were excluded. Subjects who were failing to maintain a C-average in school, had a positive history for neurologic or psychiatric disease, or a previous clinically indicated MRI scan, were excluded. Subjects were likewise excluded if they were under treatment (including medication) for any neurological or psychiatric conditions, including treatment with psychoactive drugs such as atypical stimulants, anti-depressants, or serotonin reuptake inhibitors. Additional exclusion criteria included: learning disability, head trauma with loss of consciousness, pregnancy, and birth at 37-weeks gestational age or earlier. The structural MR images obtained for each subject during this study were read by a pediatric neuroradiologist. Abnormal findings were reported to the subjects' primary physicians through an IRB-approved process. Neurocognitive assessment and testing was done under the supervision of a board certified pediatric neuropsychologist. All subjects received the Wechsler Preschool and Primary Scale of Intelligence, Revised (WPPSI-R), Wechsler Intelligence Scale for Children, Third Edition (WISC-III), or the Wechsler Adult Intelligence Scale, Third Edition (WAIS-III).

DTI data was successfully acquired from 106 children (54 F, 52 M, mean ± std age = 12.3 ± 3.5 years). The mean Full-Scale IQ score was 110.9 ± 13.7 (mean ± std). There was no significant difference between boys and girls on age (girls = 12.4 ± 3.5 years [range 5.8–18.7 years], boys = 12.1 ± 3.6 years [range 5.7–18.3 years]; $P > 0.6$, student's t-test), Full-Scale IQ (girls = 111.1 ± 15.4, boys = 110.7 ± 11.7; $P > 0.8$, student's t-test), Verbal IQ (girls = 112.2 ± 15.2, boys = 111.3 ± 12.1; $P > 0.7$, student's t-test), or Performance IQ (girls = 107.9 ± 16.1, boys = 108.3 ± 11.7; $P > 0.8$, student's t-test). Additionally, none of the three IQ measures displayed a significant sex–age interaction, examined via a two-way ANOVA ($P > 0.4$). Ninety-nine of the subjects were right-handed, six were left-handed (4 M, 2 F), and one (M) was ambidextrous according to the Edinburgh Handedness Inventory [Oldfield, 1971]. The distribution of non-right-handedness did not differ significantly between boys and girls ($P > 0.2$, χ^2 contingency test).

Scans were acquired on a Bruker 3T Medspec 30/60 MRI system with ±40 mT/m model SK330 asymmetric imaging gradients (Bruker BioSpin MRI GmbH, Ettlingen, Germany). EPI-DTI scan parameters were: TR = 6,070 ms, TE = 87 ms, FOV = 19.2 cm × 25.6 cm, slice thickness = 5 mm, matrix = 64 × 128, Δ = 40 ms, δ = 18 ms, diffusion gradient strength = 30 mT/m, b-value = 710 s/mm^2. For 47 subjects, the FOV in the readout (L-R) direction was 25.6 cm instead of 19.2 cm. Three scans were acquired without diffusion weighting, and 25 diffusion-weighted scans were acquired, with diffusion directions determined using the electrostatic repulsive model [Jones et al., 1999]. Geometric distortion due to gradient eddy currents was minimized using an automated gradient preemphasis adjustment routine [Schmithorst and Dardzinski, 2002]. The raw DTI datasets were corrected for geometrical distortion arising from main magnetic field inhomogeneity using the multiecho reference method [Schmithorst et al., 2001]. In addition, T1-weighted whole-brain 3D MP-RAGE anatomical scans were acquired for each subject. Scan parameters for the MP-RAGE scan were: TI/TR/TE = 550/15/4.5 ms, FOV = 19.2 cm × 25.6 cm × 19.2 cm, matrix = 128 × 256 × 128.

Spatial normalization and whole-brain segmentation was performed for each subject using procedures in SPM5 (Wellcome Dept. of Cognitive Neurology, London, UK) applied to the T1-weighted anatomical images. Pediatric templates for prior probabilities of gray and white matter distribution [Wilke et al., 2003a] were used to improve segmentation accuracy of our pediatric images. Unlike results obtained using an adult template, neither affine scaling parameters nor white matter probability maps correlate with age using a pediatric template [Wilke et al., 2002]. The segmentation results were output in native space and the spatial transformation into standardized Montreal Neurological Institute (MNI) space was found by normalizing the white matter probability maps to the white matter pediatric template. This procedure was used to ensure maximum accuracy for normalization of the white matter. The white-matter probability maps from each subject were then transformed into the MNI space, and resampled to 2-mm isotropic resolution. (The transformation parameters found for normalization of the white matter were also used for normalization of the DTI parametric maps.)

While the SPM5 segmentation algorithm contains built-in procedures for bias regularization (from artifacts due to B1 inhomogeneity) and for ignoring the skull (obviating the need for skull-stripping prior to normalization or segmentation) it was found that this procedure failed to yield robust results in 13 of the subjects, where the B1 inhomogeneity was especially severe. Hence the segmentation was

repeated, using the bias-corrected T1-weighted image as the starting image; this procedure yielded acceptable results in 12 of 13 subjects; in the other subject, acceptable results were obtained via skull-stripping the image using the brain extraction tool (BET) available in MRIcro (www.mricro.com) prior to the segmentation.

To minimize artifacts from subject motion, the Robust EStimation of Tensors by Outlier REjection (RESTORE) method was utilized [Chang et al., 2005]. Empirical visual analysis showed the method to be robust in rejecting data points corrupted by gross motion or by motion during the diffusion-sensitizing gradient. Visual analysis was also used to inspect the data for gross head motion (causing misregistration); this resulted in 9 data sets being rejected (not included in the count of 106 total subjects). Estimates of subject motion were also obtained from the four fMRI paradigms performed during the same scanning session as the DTI acquisition. The median voxel displacement (in mm) was computed for each subject across the four paradigms. Since subjects are likely to exhibit increased motion towards the end of the paradigms, the analysis was also repeated using only the first 2:48 of data (corresponding to the total scantime for the DTI acquisitions), and using only the first 2:00 of data in each paradigm.

The DTI tensor components were computed from each DTI dataset using the RESTORE technique. Fractional anisotropy (FA) and mean diffusivity (MD) maps were then computed from the tensor components. The maps were transformed into MNI space (using the same transformation parameters as found from normalization of the white matter) and resampled to 2 mm isotropic resolution using nearest-neighbor sampling. For additional accuracy (in case of slightly different subject positioning due to motion in the time between the DTI and whole-brain acquisitions), the FA maps were co-registered (using a rigid-body transformation) to the white matter probability maps for each subject and the MD maps were co-registered using the same transformation parameters found from the FA co-registration. For each subject, analysis was restricted to voxels with a white matter posterior probability of >0.9 from the SPM segmentation results, as well as FA >0.25. Globally, analysis was restricted to voxels in which the above criteria were met for at least 60 subjects. A total of 24,278 voxels met the criteria and were retained for further analysis. The strict thresholds used for restricting the subset of voxels analyzed minimize the risk of spurious results because of partial volume effects and imperfect spatial normalization, at the cost of only being able to examine the larger white matter tracts.

The study involved data acquired from the summer of 2001 until the winter of 2004. In September 2001, the electronics, including gradient amplifiers, on the Bruker 3T system were upgraded to the AVANCE platform; however, data from 42 subjects was acquired prior to the upgrade. Differences in gradient performance associated with the upgrade could impact the calculated FA values, and especially MD values, since the computed *b*-value is proportional to the square of the gradient amplitude. Differences in acquired resolution (due to the differing acquired voxel size in the L-R dimension) could also possibly introduce a systematic bias in the FA and MD values. We tested these potential confounding variables and found the relationship between sex and scanner architecture to be significant ($P < 0.01$, χ^2 contingency test). The relationship between sex and voxel size almost approached significance ($P = 0.053$, χ^2 contingency test). Hence the following modified General Linear Model (GLM) was used to analyze the data, to remove any possible effect because of voxel size or scanner configuration. The data was segregated into three groups: smaller voxel size and original scanner architecture, smaller voxel size and upgraded scanner architecture, and larger voxel size and upgraded scanner architecture. (There was only one subject with larger voxel size and original scanner architecture; this data was discarded.) The GLM was performed independently on the three groups and the results combined by averaging the (signed) *R*-squared values, weighted by the number of degrees of freedom in each analysis.

The modified GLM was then used to analyze the data. Three covariates were used: sex, age (in months), and sex–age interaction. The GLM was performed for the contrasts of main effect of sex, and the sex–age interaction, with age a covariate of no interest. *T*-score maps from the GLM were converted into *Z*-score maps, and filtered with a Gaussian filter of width 3 mm. However, to prevent "bleeding" of regions with significant effects into surrounding gray matter or CSF, the filtering was restricted to voxels inside the white matter mask [Schmithorst et al., 2005]. A threshold of $Z = 9$ ($Z = 10$ for MD) with spatial extent threshold of 80 voxels ($\sim$0.60 cc) was used. Since the filter width used was not significantly bigger than a single voxel, however, standard Gaussian random field theory would not provide a sufficiently accurate estimate of corrected *P*-values (for comparisons involving multiple voxels). Hence a Monte Carlo simulation, based on the method of [Ledberg et al., 1998], was used to estimate corrected *P*-values. Noise images were created from Principal Component Analysis of the data, and used to estimate the intrinsic spatial autocorrelations. The Monte Carlo simulation was performed, using those parameters, to simulate random noise with the same characteristics as present in the data. The cluster statistics from the Monte Carlo simulation were stored and used to estimate the significance of the found clusters in the data. A double-tailed threshold of $P < 0.01$ was used for significance.

For each region found to exhibit a significant main effect of sex or sex–age interaction for FA or MD, the average values (FA or MD) for the ROI were computed for boys and girls, separated out into pre-adolescent ($\leq$10 years), early adolescent ($>$10 years and $<$14 years), and late adolescent ($\geq$14 years). The centroid of each region was also computed; a transformation from MNI coordinates to Talairach coordinates was performed using the nonlinear **mni2tal** procedure outlined in (http://www.nil.

wustl.edu/labs/kevin/man/answers/mnispace.html). For the sex–age interaction, the correlation coefficients between average FA or MD and age were computed, separately for boys and girls. For each comparison (FA main effect, FA sex–age interaction, MD main effect, and MD sex–age interaction) the effect size f^2 was computed [Cohen, 1988]. Results of these analyses are included in Tables I–IV. For the regions with main effect of sex on FA or MD, the results could be affected by earlier sexual maturation in girls. (This is not a concern for the interaction effects as the differences in slopes of MA or FD with age will not be affected.) Thus, the effect sizes for the main effects were re-computed with 24 months added to the girls' ages to account for their earlier maturity (data not shown).

Results from each ROI were statistically validated via a Monte Carlo simulation as follows. The average FA or MD values were entered into a GLM (segregated by voxel size and scan group) in a similar manner as the voxelwise analysis. The Z-scores from this analysis were compared to those generated from a Monte Carlo simulation. The Monte Carlo technique simulates the distribution of Z-scores that would be obtained from spurious clusters (false positives) in the voxelwise analyses. Gaussian random noise was generated, with the same autocorrelative structure as that in the data. The dataset was then Gaussian filtered with a width of 1 voxel, to simulate the filtering applied to the Z-score maps from the voxelwise analyses. A cluster of voxels in the simulated dataset was found using a cluster size (spatial extent threshold) of 80 voxels, and an intensity threshold corresponding to the maximum value for which a cluster of 80 voxels was present. The average intensity of the voxels from the unfiltered dataset was stored and the simulation repeated 2,000 times. The Z-scores from the ROI GLM analysis were then compared to the null distribution generated from the Monte Carlo simulation for significance. A significant finding from the Monte Carlo analysis yields additional confidence in the validity and robustness of the results from the voxelwise analyses and limits the possibility of Type I errors.

Additional post-hoc analyses were also performed. ROIs with a significant main effect of sex on FA were tested for a significant main effect of sex on MD; ROIs with a significant sex–age interaction on FA were tested for a significant sex–age interaction on MD; and ROIs with a significant main effect or sex–age interaction on MD were likewise tested for similar effects on FA.

It was also desired to analyze to what extent the sex-related differences seen in FA and MD values might be associated with global white matter maturational processes, which would be reflected in global white matter volume. Age has been shown to correlate both with regional FA values [Schmithorst et al., 2002] and global white matter volume [Courchesne et al., 2000] in the age range under study, indicating that age might be a suitable proxy for global white matter maturation. However, there still might be sex-related differences from differing developmental trajectories in global white matter volume between boys and girls. Because of time constraints, we used a fast scan sequence for acquiring our whole-brain anatomical data, which, while providing a reasonable measure of white matter location and probability using SPM, was nevertheless not of sufficient quality to enable a reliable quantification of cerebral global white matter volume, as there was a significant confound from the failure of the SPM procedure to remove extra-cerebral tissue, and to reliably segment areas of white matter (such as the internal capsule) from surrounding gray matter in which there was low contrast (such as the thalamus and basal ganglia). Hence, global white matter volume was excluded from subsequent analyses.

RESULTS

The stringent criteria used for the global white matter mask resulted in inclusion of most areas of white matter, with the exception of the body of the corpus callosum near midline and the most frontal and lateral aspects of the arcuate fasciculus bilaterally (Fig. 1). The nonlinear normalization technique used was robust for most white matter areas; however, there was significant inter-subject variability in the morphometry of the superior region of the corpus callosum. The most frontal and lateral aspects of the arcuate fasciculus were also excluded because of the smaller size of the white matter tracts in this region; analyzing smaller tracts with the acquired voxel size (3 mm × 2 mm × 5 mm or 4 mm × 2 mm × 5 mm) will result in partial-volume effects and contamination with surrounding gray matter being a substantial confound.

Results from the motion analysis performed on the fMRI data yielded significantly more motion in boys than in girls ($P < 0.05$, independent t-test). The mean (±std.) displacement in boys was 1.67 ± 0.89 mm and the mean (±std.) displacement in girls was 1.30 ± 0.86 mm. When the motion analysis was repeated using only data from the start of the fMRI paradigms up to the total scantime for the DTI acquisition (2:48), the results improved. The mean (±std.) displacement in boys was 1.22 ± 0.75 mm and the mean (±std.) displacement in girls was 0.95 ± 0.64 mm.

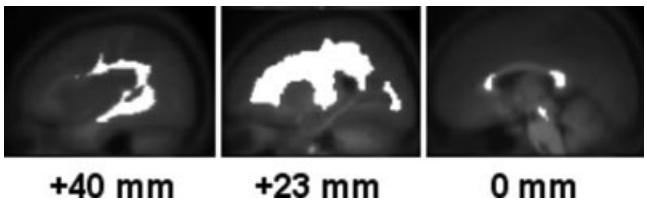

Figure 1.
The white matter mask (pure white voxels), displaying the voxels used for analysis of the DTI data (overlaid on the averaged whole-brain anatomical image for selected sagittal slices). Slice location (L-R; Talairach coordinate system) given at bottom of each frame.

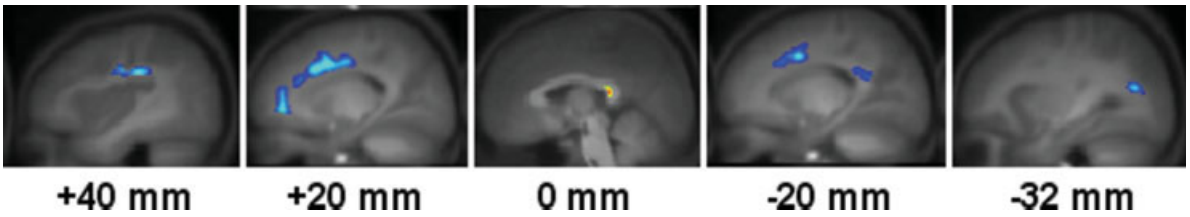

Figure 2.
Regions with a significant main effect of sex on FA (blue = boys > girls, yellow-red = girls > boys) in a cohort of 105 children ages 5–18 years. Slice location (L-R; Talairach coordinate system) given at bottom of each frame.

The difference was also significant ($P < 0.05$, independent t-test). Repeating the analysis using only data from the start of the fMRI paradigms until 2:00 elapsed in the paradigms, the mean (± std.) displacement in boys was 1.06 ± 0.70 mm and the mean (± std.) displacement in girls was 0.84 ± 0.58 mm. This difference is no longer significant ($P > 0.05$, independent t-test).

Results for main effect of sex on FA are displayed in Figure 2. Average FA values for each region found are tabulated in Table I, separated out by sex and age grouping (pre, early, and late adolescence). Girls displayed higher FA than boys in the splenium of the corpus callosum. Boys displayed higher FA than girls in frontal white matter areas bilaterally, in the right arcuate fasciculus, and in left parietal and occipito-parietal white matter.

Results for the sex–age interaction on FA are displayed in Figure 3. Average FA values for each region are tabulated in Table II, again separated out by sex and age group. The correlation coefficients of age with FA are tabulated for each region separately for boys and girls. A threshold of $|R| > 0.27$ (corresponding to $P < 0.05$ double-tailed) is used to indicate significant correlation with age. Boys displayed a positive correlation of FA with age and girls displayed a negative correlation of FA with age in the left frontal lobe. Girls displayed a positive correlation of FA with age and boys displayed a negative correlation of FA with age in the right arcuate fasciculus. Girls display a positive correlation of FA with age, with no significant correlation in boys, in the right frontal lobe and right occipito-temporo-parietal white matter.

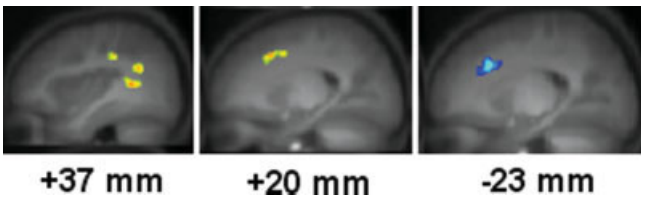

Figure 3.
Regions with a significant sex–age interaction on FA (blue = boys > girls, yellow-red = girls > boys) in a cohort of 105 children ages 5–18 years. Slice location (L-R; Talairach coordinate system) given at bottom of each frame.

Results for main effect of sex on MD are displayed in Figure 4 and tabulated in Table III. Boys displayed higher MD in the corticospinal tract bilaterally and in the right frontal lobe. Girls displayed higher MD in the right arcuate fasciculus, right occipito-parietal white matter, and most superior aspect of the corticospinal tract in the right hemisphere.

Results for the sex–age interaction on MD are displayed in Figure 5 and tabulated in Table IV. Girls displayed a negative correlation with age in the frontal lobes bilaterally, and in the right arcuate fasciculus and occipito-parietal areas. Boys displayed no significant correlation in the left frontal lobe and a significant negative correlation in the right hemisphere (but with a lesser correlation compared to girls).

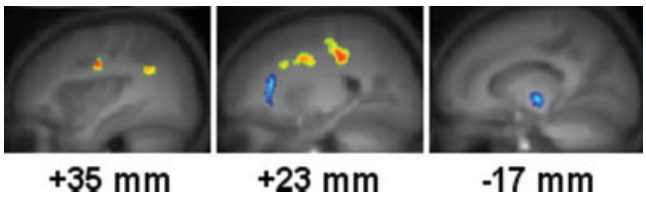

Figure 4.
Regions with a significant main effect of sex on mean diffusivity (MD) (blue = boys > girls, yellow-red = girls > boys) in a cohort of 105 children ages 5–18 years. Slice location (L-R; Talairach coordinate system) given at bottom of each frame.

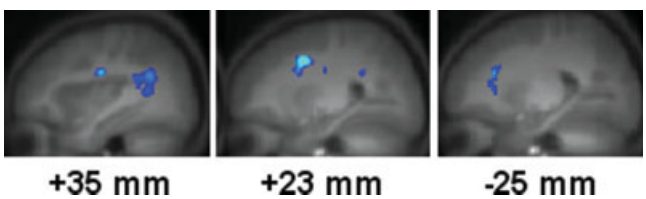

Figure 5.
Regions with a significant sex–age interaction on mean diffusivity (MD) (blue = boys > girls) in a cohort of 105 children ages 5–18 years. Slice location (L-R; Talairach coordinate system) given at bottom of each frame.

TABLE I. Regions with significant differences in fractional anisotropy (FA) between boys and girls in a sample of 105 children ages 5–18 years and the effect size (Cohen's f^2)

X, Y, Z	No. of voxels	Location	F^2	Pre boys	Pre girls	Early boys	Early girls	Late boys	Late girls
−37, −55, 21	239	LOccParietal	0.22	0.48 ± 0.011	0.45 ± 0.008	0.49 ± 0.007	0.48 ± 0.011	0.51 ± 0.011	0.47 ± 0.009
20, 24, 23	1,018	RFrontal	0.27	0.42 ± 0.007	0.41 ± 0.006	0.44 ± 0.004	0.42 ± 0.006	0.45 ± 0.005	0.42 ± 0.006
−18, −46, 26	100	LParietal	0.09	0.55 ± 0.016	0.51 ± 0.013	0.55 ± 0.019	0.54 ± 0.016	0.56 ± 0.016	0.54 ± 0.014
40, −19, 32	225	RArcuate	0.28	0.46 ± 0.007	0.43 ± 0.010	0.47 ± 0.007	0.46 ± 0.007	0.51 ± 0.009	0.46 ± 0.008
−21, 13, 36	246	LFrontal	0.18	0.44 ± 0.008	0.43 ± 0.006	0.45 ± 0.007	0.43 ± 0.008	0.46 ± 0.009	0.43 ± 0.008
−1, −34, 18	118	Splenium	0.17	0.72 ± 0.027	0.80 ± 0.018	0.79 ± 0.014	0.84 ± 0.012	0.82 ± 0.012	0.84 ± 0.013

Location (X, Y, Z) in Talairach coordinates. FA values (mean ± SEM) are broken down by age into pre-adolescent (≤10 years), late adolescent (≥14 years), and early adolescent (>10 years and <14 years). OccParietal, occipito-parietal white matter.

The effect sizes (Cohen's f^2) for each contrast (main effect of FA, main effect of MD, sex–age interaction on FA, sex–age interaction on MD) are also displayed in Tables I–IV and range from 0.09 to 0.37. As described in the Materials and Methods section, effect sizes were also computed for the main effects of FA and MD when the girls' ages were corrected for their earlier maturity. For the main effect of FA, correcting the girls' ages for maturity resulted in a larger effect size for every region with the exception of the splenium, where f^2 was reduced from 0.17 to 0.09. The FA difference for most of the regions (boys > girls) is opposite to what would be expected from girls' earlier maturity, since FA has been shown to increase with age in the white matter during normal development [Schmithorst et al., 2002]. For the main effect of MD, correcting the girls' ages for maturity resulted in a larger effect size for the right occipito-parietal, right arcuate fasciculus, and most superior aspect of the right corticospinal tract; approximately the same effect size for the corticospinal tract bilaterally; and a reduction in effect size for the right frontal lobe (f^2 reduced from 0.13 to 0.04). This is related to the decrease of MD with age in the white matter during normal development; the MD differences for the three regions with girls > boys is opposite to what would be expected from girls' earlier maturity. There is a slight decrease of effect size in the corticospinal tract (where MD of boys > girls); this decrease is only slight since the magnitude of the sex-related difference is significantly larger than the magnitude of the age-related change. However, a large decrease in effect size was seen in the right frontal lobe and thus this result must be viewed with caution as it could be confounded with effects because of girls' earlier maturation.

For each region exhibiting a significant sex–age interaction effect for FA, scatter plots are shown in Figure 6. For each region displaying a significant sex–age interaction effect for MD, scatter plots are shown in Figure 7. The plots graphically illustrate the differences in developmental trajectories between boys and girls for these regions. A summary of the main findings of FA in the frontal lobes is shown in Figure 8, with regions exhibiting a significant main effect of sex on FA shown together with regions exhibiting sex–age interactions.

For the validation procedure using the Monte Carlo simulation, the null distribution of Z-scores had a mean ± std. of 2.08 ± 0.19. Each ROI found significant from the voxelwise analysis had a (absolute value) Z-score from the post-hoc GLM analysis of 2.94 or larger, corresponding to double-sided $P < 0.001$, with the exception of the left parietal region exhibiting a main effect of sex on FA, which had a Z-score of 2.57, corresponding to double-sided $P < 0.025$.

For the post-hoc analyses (testing regions found via the voxelwise analysis with a main effect or sex–age interaction on FA for similar effects on MD and vice versa) the results are as follows. All regions with a significant main effect of sex on FA (Table I) also exhibited a significant ($P < 0.05$) main effect on MD; the splenium had greater

TABLE II. Regions with significant sex-age interactions in fractional anisotropy (FA) between boys and girls in a sample of 105 children ages 5–18 years and the effect size (Cohen's f^2)

X, Y, Z	No. of voxels	Location	F^2	BoyR	GirlR	Pre boys	Pre girls	Early boys	Early girls	Late boys	Late girls
−24, 21, 29	266	LFrontal	0.27	0.48	−0.38	0.40 ± 0.007	0.42 ± 0.006	0.41 ± 0.007	0.41 ± 0.006	0.43 ± 0.009	0.39 ± 0.005
34, −50, 21	241	ROccTempPar	0.37	−0.22	0.68	0.48 ± 0.008	0.45 ± 0.010	0.48 ± 0.007	0.49 ± 0.009	0.48 ± 0.009	0.51 ± 0.006
33, −31, 36	85	RArcuate	0.12	−0.40	0.28	0.49 ± 0.009	0.47 ± 0.010	0.48 ± 0.010	0.48 ± 0.013	0.46 ± 0.013	0.47 ± 0.011
19, 15, 39	110	RFrontal	0.17	0.07	0.51	0.46 ± 0.015	0.42 ± 0.009	0.47 ± 0.007	0.43 ± 0.013	0.47 ± 0.014	0.48 ± 0.010

Location (X, Y, Z) in Talairach coordinates. R values, correlation coefficients between FA and age in boys and girls. FA values (mean ± SEM) are broken down by age into pre-adolescent (≤10 years), late adolescent (≥14 years), and early adolescent (>10 years and <14 years). OccTempPar, occipito-temporo-parietal white matter.

TABLE III. Regions with significant differences in mean diffusivity (MD) between boys and girls in a sample of 105 children ages 5–18 years and the effect size (Cohen's f^2)

X, Y, Z	No. of voxels	Location	F^2	Pre boys	Pre girls	Early boys	Early girls	Late boys	Late girls
−15, −19, −1	373	CST	0.24	7.66 ± 0.117	7.39 ± 0.157	7.72 ± 0.122	7.38 ± 0.131	7.73 ± 0.116	7.19 ± 0.133
22, 31, 12	132	RFrontal	0.13	8.47 ± 0.129	7.88 ± 0.132	8.46 ± 0.363	7.87 ± 0.165	7.84 ± 0.169	7.39 ± 0.100
36, −52, 31	80	ROccParietal	0.13	8.23 ± 0.189	8.57 ± 0.180	7.83 ± 0.151	8.04 ± 0.112	7.36 ± 0.157	7.70 ± 0.103
23, 3, 35	463	RArcuate	0.13	7.91 ± 0.082	7.96 ± 0.097	7.62 ± 0.061	7.80 ± 0.097	7.43 ± 0.084	7.58 ± 0.064
23, −26, 45	310	RSupCST	0.10	7.91 ± 0.106	7.95 ± 0.080	7.61 ± 0.060	7.74 ± 0.079	7.36 ± 0.080	7.57 ± 0.078

Location (*X*, *Y*, *Z*) in Talairach coordinates. MD values ($\times 10^{-4}$ mm^2/s; mean ± SEM) are broken down by age into pre-adolescent (≤10 years), late adolescent (≥14 years), and early adolescent (>10 years and <14 years). CST, cortico-spinal tract, OccParietal, occipito-parietal white matter.

MD in boys while all other regions showed greater MD in girls. Of the regions with a significant sex–age interaction effect on FA (Table II), only the right occipito-temporo-parietal region and the right arcuate fasciculus exhibited a significant sex–age interaction effect on MD (boys > girls). For the regions with a significant main effect of sex on MD (Table III), all regions exhibited a significant main effect of sex on FA with the exception of the right frontal lobe; the corticospinal tract exhibited greater FA in girls while the other regions (right arcuate fasciculus, right occipito-parietal white matter, and the most superior aspect of the corticospinal tract in the right hemisphere) exhibited greater FA in boys. Of the regions with a significant sex–age interaction effect on MD (Table IV), all regions except the left frontal region exhibited a significant sex–age interaction effect on FA (girls > boys).

DISCUSSION

Subject Population, Effect Sizes, and Study Power

Since all subjects were recruited for a normal cross-sectional study of language development and scored within the normal range for neurological and neuropsychological measures, and their whole-brain scans were read as normal, our results may be taken as representative of a normal population, avoiding possible issues with transferability of results [Rivkin, 2000] for studies involving clinically referred subject populations only retrospectively classified as normal. We expected the magnitude of the differences to be small, and sensitivity was further reduced via use of a voxelwise analysis technique (necessary due to the lack of an a priori hypothesis). Thus, we utilized a rather large sample size (>100 subjects) in order to achieve sufficient power to detect differences between boys and girls.

Using a freely-available software program, G*Power 3.0.3 [Faul et al., 2007], a "sensitivity power analysis" was conducted in order to determine theoretically derived (minimum detectable) population effect sizes. For a population of 100 subjects, an effect size f^2 of 0.08 is detectable with $\alpha = 0.05$ and power = 0.8, and this approximately corresponds to the lowest effect size found in our analysis. However, most regions detected in our analysis had larger effect sizes. With 60 subjects an effect size of $f^2 = 0.13$ would be detectable at the same α and power level. Thus, studies with ≥60 subjects would be expected to detect most of the regions found in the current study. Improved sensitivity is also likely if an ROI-based DTI analysis technique (incorporating a priori hypotheses) is used in preference to a voxelwise analysis technique, and thus our results indicate that future DTI studies of sex differences may be feasible with somewhat more moderate sample sizes.

Physiologic Correlates of FA and MD Differences

Differences in FA or MD could reflect a number of possible physiologic differences in the brain [Schmithorst et al., 2002], and the exact physiological correlates of differences in

TABLE IV. Regions with significant sex-age interactions in mean diffusivity (MD) between boys and girls in a sample of 105 children ages 5–18 years and the effect size (Cohen's f^2)

X, Y, Z	No. of voxels	Location	F^2	Boy R	Girl R	Pre boys	Pre girls	Early boys	Early girls	Late boys	Late girls
−28, 25, 22	231	Lfrontal	0.19	−0.21	−0.60	7.87 ± 0.103	7.99 ± 0.130	7.89 ± 0.098	7.80 ± 0.079	7.70 ± 0.101	7.52 ± 0.101
34, −49, 25	365	ROccParietal	0.18	−0.38	−0.65	8.54 ± 0.138	8.76 ± 0.145	8.35 ± 0.103	8.17 ± 0.117	8.21 ± 0.126	7.96 ± 0.093
33, −11, 29	80	RArcuate	0.14	−0.26	−0.61	7.58 ± 0.117	7.80 ± 0.099	7.39 ± 0.080	7.44 ± 0.136	7.38 ± 0.130	7.19 ± 0.110
24, 8, 35	246	Rfrontal	0.13	−0.39	−0.57	7.85 ± 0.072	7.97 ± 0.108	7.69 ± 0.066	7.74 ± 0.124	7.56 ± 0.093	7.51 ± 0.077

Location (*X*, *Y*, *Z*) in Talairach coordinates. *R* values, correlation coefficients between MD and age in boys and girls. MD values ($\times 10^{-4}$ mm^2/s; mean ± SEM) are broken down by age into pre-adolescent (≤10 years), late adolescent (≥14 years), and early adolescent (>10 years and <14 years). OccParietal, occipito-parietal white matter.

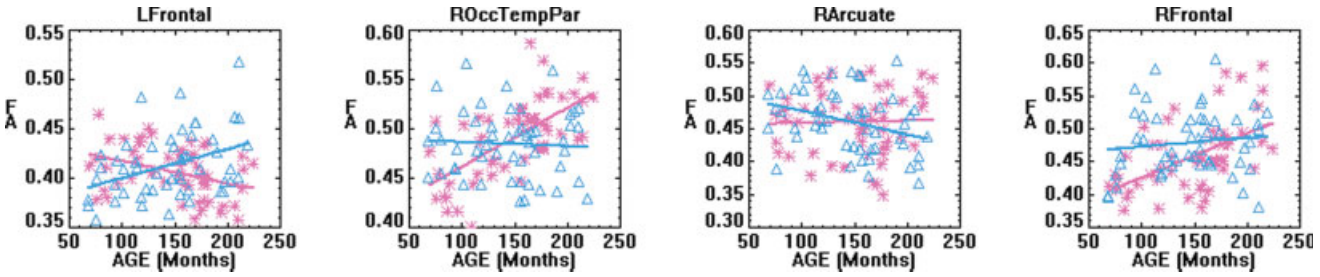

Figure 6.
Scatterplots of FA for girls (pink, asterisks) and boys (blue, triangles) versus age, for each region displaying a significant sex–age interaction (Table II).

FA and/or MD are a burgeoning topic of research which we do not propose to address here. Differences in FA are mostly thought to be the result of differences in fiber organization, but could also be related to myelination, fiber density, axonal diameter, and ratio of intracellular/extracellular space. Differences in MD mostly relate to fiber density, but are also affected by differences in axonal diameter and myelination. Differences in the intracellular fraction relative to the extracellular fraction are unlikely to result in a substantial difference in MD [Duong et al., 2001].

Developmentally, decreases in MD with age and increases in FA with age were shown throughout the white matter [Schmithorst et al., 2002] in the same age range as used in the present study. Thus, our results suggest differences in maturational processes (affecting fiber density, myelination, organization, and/or axonal diameter) between boys and girls. Myelination differences in particular may have important developmental implications; even though the brain is mostly myelinated by age 5, continuing myelination during the developmental period may be an important developmental process [Fields, 2005], even possibly extending to age 30.

Regions With FA Differences Between Boys and Girls

The splenium of the corpus callosum (Table I) was found to exhibit a significant main effect of FA with girls > boys. Via a post-hoc analysis, this region also showed a significant sex–age interaction effect ($T(90) = -2.6$, $P < 0.05$), with boys showing a positive correlation with age and "catching up" to girls later in development. Our results thus indicate that organization of the splenium fiber tracts occurs later in boys than in girls. The splenium may in fact continue to mature in young adult men, as all areas of the corpus callosum including the splenium were shown to have greater FA in adult men as compared to adult women [Westerhausen et al., 2004]. It has been hypothesized [Westerhausen et al., 2004] that the greater FA in adult men in the corpus callosum is due to the corpus callosum in males containing fewer but thicker myelinated fibers. A histological study reported that male rats exhibit thicker myelin sheaths in the splenium compared to females [Kim et al., 1996]. Our results are consistent with a process of increasing myelination in the splenium of the corpus callosum in boys. The post-hoc analysis showed greater MD ($P < 0.05$) in the splenium for boys compared to girls, showing lower fiber density in males.

In accordance with our hypothesis, some associative white matter regions were found to exhibit greater FA in boys than girls (Table I), including frontal regions bilaterally, the right arcuate fasciculus, and left parietal and occipito-parietal white matter. This sex-related difference appears to be present across the age range (Table I). Smaller FA in females in associative regions may appear at first glance to be contrary to previous findings indicating

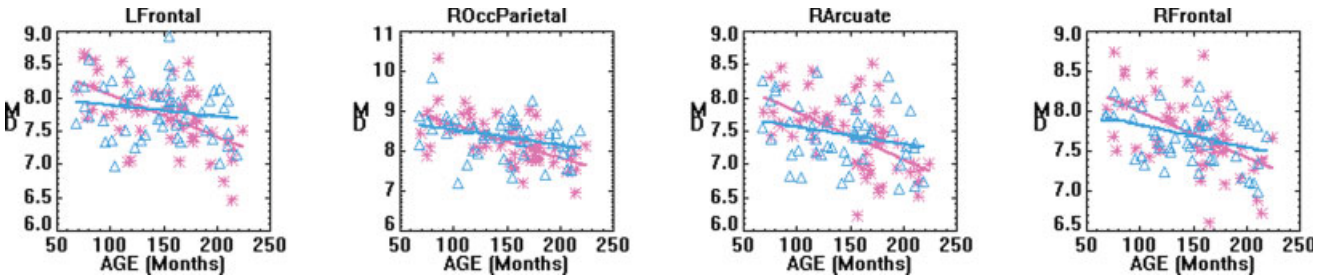

Figure 7.
Scatterplots of MD ($\times 10^{-4}$ mm^2/s) for girls (pink, asterisks) and boys (blue, triangles) versus age, for each region displaying a significant sex–age interaction (Table IV).

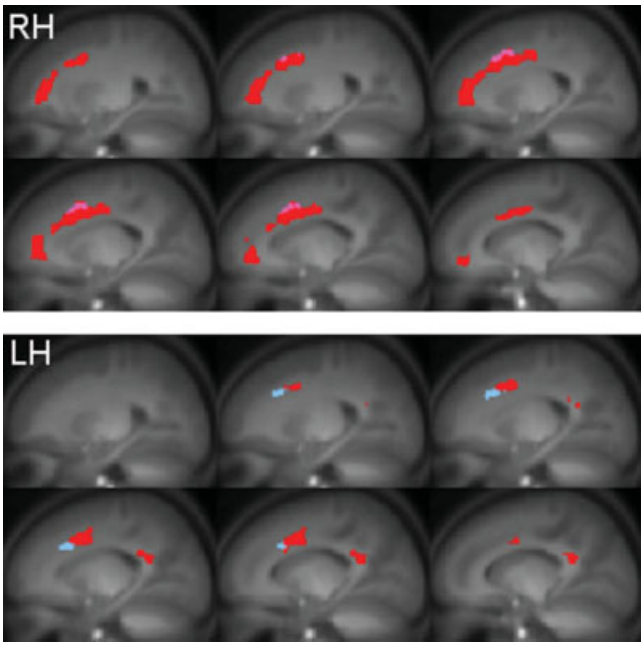

Figure 8.
Illustration of sex and hemispheric differences for developmental changes in FA in the frontal lobes (top, right hemisphere; bottom, left hemisphere). Colored voxels (all colors) indicate FA boys > FA girls (main effect). Blue voxels indicate sex–age interaction (boys > girls); pink voxels indicate sex–age interaction (girls > boys). Slice locations (both hemispheres, Talairach coordinates): $|X| = 15$ mm (top-left) to $|X| = 25$ mm (bottom-right).

that women possess a greater number of neuronal processes [de Courten-Myers, 1999] and a greater dependence on white matter architecture and/or organization for cognitive function [Gur et al., 1999; Haier et al., 2005; Jung et al., 2005; Schmithorst and Holland, 2006; Schmithorst et al., 2005], as well as smaller white matter volume. However, in the post-hoc analysis all of the ROIs showing FA in boys > FA in girls (Table I), also showed significantly ($P < 0.05$) higher MD values in girls compared to boys. Thus we hypothesize that the more constrained white matter space, lower degree of fiber density, and greater dependence on intra- and inter-hemispheric connectivity in females necessitates increased crossing of white matter fiber tracts, resulting in lower FA values. Further research will be necessary to determine whether the FA differences found in fact indicate different developmental patterns of white matter organization between girls and boys; our results provide preliminary support for such a viewpoint.

Our results in the frontal lobes might seem to conflict with a previous study [Szeszko et al., 2003] in which greater FA was shown in the frontal lobes in adult women. This discrepancy may be the result of continued maturation of the frontal lobes beyond the oldest age in our study, as the average age of subjects in the Szeszko et al. study was approximately 30 years, and the peak of white matter volume has been shown to occur at around 45–50 years of age [Bartzokis et al., 2001]. The frontal lobes are the latest to develop [Wilke et al., 2003b], and delayed development in boys may result in later pruning of anterior white matter tracts after the age of 18; further research will be necessary to investigate development of the frontal lobes in the young adult age range. The discrepancy may also be related to intelligence; measures of general intelligence are not given in the Szeszko et al. study. Differing trends in functional lateralization with age in children and young adults as compared with older adults [Szaflarski et al., 2006] have been demonstrated, which suggests that pruning and optimization of neural circuits may differ among these age groups.

Regions With Sex–Age Interactions on FA

One region was found in the left frontal lobe (Fig. 3; Table II) showing a significant sex–age interaction effect on FA with boys > girls. (The sex–age interaction effect is interpreted as differences between boys and girls in the slope of FA as a function of age. Accordingly, in this region boys displayed a positive correlation of FA with age while girls displayed a negative correlation of FA with age (Fig. 3; Table II).) The post-hoc analysis for this region did not reveal a significant sex–age interaction with MD. Hence, the FA and MD results may indicate increased white matter organization in boys (compared to girls) as a function of age in the left inferior frontal gyrus, but with no difference in the decrease of white matter density in boys as a function of age.

This developmental framework is supported by previous structural and functional MRI studies. The left inferior frontal gyrus has been identified as a region in which boys persist in maintaining greater relative gray matter volume when compared with girls [Blanton et al., 2004], in contrast to the global trend in the brain [De Bellis et al., 2001] of boys' decreasing relative gray matter volume to a lower level than girls by age 12, continuing into early adulthood. An fMRI study [Schmithorst and Holland, 2007] has shown a positive correlation of intelligence in boys with functional connectivity involving the left inferior frontal gyrus (Broca's area) for narrative comprehension, with the opposite effect (negative correlation) present in girls. Consistent with a previously hypothesized greater dependence in boys for optimal neuronal pruning on intelligence [Haier, 1993], our results suggest that the optimal developmental strategy for boys may involve less neuronal pruning in the left frontal lobe relative to other regions together with increased connectivity with other brain regions, reflected in an increase in FA with age and a greater proportion of gray matter. On the other hand, girls' decreased reliance on functional connectivity with the left inferior frontal gyrus would reflect a decrease in FA; unnecessary or inefficient fiber tracts would no longer be maintained, as inefficient synaptic connections are removed through the gray matter pruning process [Casey et al., 2000;

Courchesne et al., 2000; Huttenlocher and Dabholkar, 1997].

Our results in the left inferior frontal gyrus, however, are also consistent with greater myelination in boys during development. As adult men have fewer neuronal processes [de Courten-Myers, 1999], they may be more reliant on the greater speed in neuronal connections which increased myelin provides. These differences may result from hormonal differences during development [Ducharme and Forest, 1993], stronger hemispheric specialization in males [Hiscock et al., 1994], or other factors. As this region is adjacent to classical language processing areas (BA 44/9), our results, showing continued maturation and directional organization of left frontal white matter areas in boys but not in girls, are in line with a recent study [Pujol et al., 2006] postulating that myelination in infants and toddlers is a critical step in the development of language skills. It is well known that the acquisition of certain language skills is delayed in boys relative to girls, and our results show continued maturation of left frontal white matter in boys, which may relate to continuing myelination.

Three regions in the right hemisphere (Table II; Fig. 3) showed a significant sex–age interaction on FA with girls > boys (slope of FA vs. age greater in girls). Girls display increasing FA with age in regions in the right hemisphere, with boys either showing no correlation or a decreasing correlation of FA with age (Figs. 6 and 8). The post-hoc analysis showed a significant ($P < 0.05$) age–sex interaction in MD (boys > girls, indicating a greater rate of decrease of MD in girls) for the right occipito-temporo-parietal region and the right arcuate fasciculus; while no significant age–sex interaction was shown for the right frontal area the region has a similar location to a region displaying an age–sex interaction in the voxelwise analysis (Fig. 5, Table IV).

Thus, girls appear to display a greater rate of increase with age of fiber density, organization, and/or myelination in these right hemisphere regions. Our results are in line with a previous fMRI study [Schmithorst and Holland, 2007] showing increasing reliance for intelligence in girls on inter-hemispheric functional connectivity and intra-hemispheric functional connectivity in the right hemisphere. These results may relate to hemispheric specialization; some evidence [Hiscock et al., 1994, 1995, 2001] suggests that adult men have stronger hemispheric specialization than women for various tasks, including auditory, visual, and dual-task interference, as well as language tasks [Shaywitz et al., 1995]. Our results, with different developmental trajectories between the hemispheres, provide preliminary support of a sex-hemisphere interaction effect in brain development.

Regions With MD Differences Between Boys and Girls

Boys displayed significantly greater MD (reflecting reduced fiber density), in the corticospinal tract at the level of the internal capsule bilaterally, and the right frontal lobe; while girls displayed significantly greater MD in the right arcuate fasciculus, right occipito-parietal white matter, and the most superior aspect of the corticospinal tract in the right hemisphere (Fig. 4, Table III). These results are contrary to our hypothesis of greater MD values in boys globally (the result of greater absolute white matter volume and hence less white matter density).

Our results showed evidence of regionally specific, rather than global, differences in MD. This indicates that the differences in MD values likely reflect regionally-specific relative, rather than global absolute, white matter volume differences. An anatomical MRI study [Gur et al., 2002] found regionally-specific differences in gray matter, with women showing greater relative volume in the orbital frontal cortex but not in the prefrontal cortex; sex-related differences in relative white matter volume may exhibit similar regional specificity [Blanton et al., 2004; De Bellis et al., 2001].

In addition, three regions (right arcuate fasciculus, right occipito-parietal white matter, and most superior aspect of the right corticospinal tract) displayed smaller MD (indicating greater density) in boys, contrary to our expectation of greater density in girls. The right arcuate fasciculus and right occipito-parietal white matter also displayed significantly ($P < 0.05$) greater FA in boys via the post-hoc analysis. As stated earlier, our hypothesis is that smaller FA and higher MD in girls reflect a greater degree of white matter fiber crossing in girls, necessitated due to smaller fiber density in a more constrained space concomitant with greater dependence for cognitive function on intra- and inter-hemispheric connectivity. Our results are not consistent with myelination differences in these associative regions: a greater degree of myelination in boys would reflect in greater FA but also reduced density and higher MD values.

Greater MD was seen in the right frontal lobe in boys. This result is however confounded with delayed development in boys. The effect size was decreased when a correction was made for girls' earlier maturation (as described in the Results section). The frontal lobes are the last to develop [Casey et al., 2000; Huttenlocher and Dabholkar, 1997] and may develop later in males compared to females, with development continuing beyond the oldest age (18 years) available in the study.

The MD differences seen in the corticospinal tract could reflect differences in fiber tract size or shape. Post-mortem data from elderly subjects [Zhou et al., 2000] displayed some microstructural differences in the corticospinal tract between men and women, involving axonal shape and size. Microstructural differences in the corticospinal tract during the developmental period may be associated with differences in performance in motor tasks which favor either boys or girls depending on the specific task [Ruff and Parker, 1993].

Regions With Sex–Age Interactions on MD

A faster rate of decrease of MD with age in girls as compared to boys was seen in the right frontal lobe, and in the

right arcuate fasciculus and occipito-parietal areas (Table IV; Fig. 5), and these regions also showed a significant ($P < 0.05$) sex–age interaction in FA (girls > boys) via the post-hoc analysis. These results support the proposed sex-hemisphere interaction effect during development, with older girls increasingly relying on the right hemisphere for cognitive function, and are consistent with boys' greater increase in absolute and relative white matter volume during development [Caviness et al., 1996; De Bellis et al., 2001]. It should be pointed out that a study in older adults (mean age ~60 years) showed no significant MD differences between men and women [Ahlhelm et al., 2004], pointing to the possibility of sexually dimorphic white matter maturation beyond the 18th year of life. A future study examining the development of white matter in adults will be necessary to investigate this hypothesis further.

A possibly counter-intuitive result is the sex–age interaction effects seen in the left frontal lobe (Tables II and IV): girls display a decrease in FA but also a decrease in MD. However, the post-hoc analysis revealed neither a significant sex–age effect on MD using the ROI found with a significant sex–age effect on FA, nor a significant sex–age effect on FA using the ROI found with a significant sex–age effect on MD. Comparing the anatomical location of the ROIs, the region with a sex–age interaction on FA (Fig. 3) mainly comprises white matter adjoining the superior aspect of a classical language area (Broca's area; BA 44/9), with only a small portion of more posterior and inferior white matter. However, the region with a sex–age interaction on MD (Fig. 5) mainly comprises the fronto-occipital fasciculus and a portion of the arcuate fasciculus. These results are consistent with previous studies showing a greater reliance for intelligence in girls with age on functional connectivity in the left hemisphere generally [Schmithorst and Holland, 2006], with however a greater reliance for intelligence in boys on functional connectivity with Broca's area [Schmithorst and Holland, 2007].

The Importance of Controlling for Sex in DTI Developmental Studies

Our results provide preliminary evidence supporting the existence of regionally specific developmental differences in white matter microstructure between girls and boys. Regionally specific increases in FA [Barnea-Goraly et al., 2005; Schmithorst et al., 2002] and decreases in MD [Schmithorst et al., 2002] with age over this age range have been shown previously and hypothesized to be the result of increased fiber organization, density, and/or myelination with age. Our results now show that this process of white matter maturation may be sexually dimorphic. This finding indicates the importance of taking sex into account as a demographic variable in DTI developmental studies. Many of the interesting findings (such as development in the frontal lobes) would not be seen if the data from males and females were simply combined, as the effects from the two groups would cancel out. Moreover, our results indicate that in sex-unbalanced samples great care must be taken in extrapolating the results to more general populations.

Limitations of this Study

The study is subject to several limitations. A significant fraction of the study population underwent a slightly different DTI protocol, involving a somewhat larger voxel size in the L-R direction; moreover, in the middle of the study period a major scanner upgrade occurred. It was desired, however, to combine the data for increased statistical power. The data was therefore segregated in appropriate groups and analyzed separately; in addition, a very stringent mask threshold (posterior white matter probability >0.9 and FA >0.25) was employed, to guard against partial-volume effects from gray matter and cerebrospinal fluid. Exploratory analyses (data not shown) indicated that the larger voxel size resulted in significantly lower apparent MD values and lower apparent FA values in some regions in the white matter, likely because of white matter tortuosity or crossing fibers, which would result in lower detected FA and MD values with larger voxel sizes. Our FA and MD values for a particular brain region and subject population may only be taken as representative for a particular voxel size, though values are averaged across approximately equal numbers of subjects scanned with both protocols.

The stringent mask threshold also prevented the investigation of sex differences within smaller white matter tracts, and our relatively large voxel size would have made such an investigation problematic with partial-volume effects because of differences in morphology being a likely confound. We used a very conservative approach, to guard against spurious results from partial-volume effects and imperfect spatial normalization. We also used a stringent threshold for significance in the voxelwise analyses and validated our results using a post-hoc Monte Carlo simulation technique. Hence, we fully expect that there may be even more areas with sex-related differences in white matter maturation than those found in this study. Particularly, the main body of the corpus callosum was excluded from analysis; further research may investigate this area using a region-of-interest based analysis technique. A further possible confounder is handedness; previous studies have shown dependence of FA on handedness in regionally specific areas including the corpus callosum and superior corticospinal tract [Buchel et al., 2004; Westerhausen et al., 2003, 2004]. Since there were no significant differences between boys and girls in the incidence of non-right-handedness, and the incidence overall was within the range for a normal population, it was decided to include the non-right-handed subjects in the analysis.

For sex differences found in mean diffusivity, it is impossible to rule out differences in temperature as a possible confounding factor. Mean diffusivity is monotonically related to the temperature of the sample. Thus, our differ-

ences could be partly the result of girls having a slighter lower temperature in their brain tissue than boys. The MRI scanner is supercooled via cryogens, and this has the effect of lowering the surrounding air temperature in the scanner bore. We cannot rule out the possibility that our results partly reflect differences in thermal regulation. A study in adults found no sex-related differences in intraventricular temperature [Fountas et al., 2004]; nevertheless it is possible that such an effect exists in children. While it would appear that the differences in MD we report are significantly larger than would be expected via naïve application of the Stokes-Einstein relation for temperature differences on the order of 1°C, the temperature dependence on MD for white matter may be different than predicted via the Stokes–Einstein relation for free water.

Finally, in studies involving children, subject compliance, particularly with regard to head motion, can also become a significant issue. Head motion actually will produce two sources of artifacts: excess movement during the diffusion-sensitizing gradient will result in slice "dropout" or significant signal loss; moreover, gross motion during the duration of the scan will result in misregistration of the diffusion-weighted images. Steps taken to reduce such artifacts included visual inspection of the scans for gross motion, and the use of RESTORE, an advanced post-processing technique [Chang et al., 2005] designed to account for outliers resulting from gross motion or head motion during the diffusion-sensitizing gradients. However, we cannot discount entirely the possibility of motion artifacts affecting our results, since from the fMRI data the prevalence of motion is seen to be significantly greater in boys than in girls. However, the effect only just reached significance when the fMRI datasets were restricted to the first 2:48 of data in the fMRI paradigms (corresponding to the scantime for the DTI acquisitions) and the effect was not significant when the fMRI datasets were restricted to the first 2:00 of data. Thus, there does not appear to be a significant effect over much of the DTI acquisition, and the RESTORE technique should remove most of the datapoints corrupted by motion. The results from the FA analysis also yield a degree of confidence that our results were not overly affected by the presence of motion. More gross motion in boys would result in lower FA values in white matter tracts because of partial volume effects, whereas higher FA values were found in boys in many areas in the white matter, with only one area (the splenium) showing higher FA in girls.

Because of these limitations, and due to the remaining possibility of Type I errors in our analysis (despite our rigorous procedure involving Monte Carlo simulation for validating the corrected *P*-value) our results await independent replication. Sex differences found in exploratory analyses of fMRI data have sometimes been found not to replicate to a second study [Haut and Barch, 2006], although this difficulty may not be as relevant in DTI data, as it involves structural information alone and is not confounded by cognitive factors present in fMRI studies.

CONCLUSION

A cross-sectional DTI study was conducted on 105 children ages 5–18 years. Regionally specific differences were found in FA and MD between girls and boys. Girls displayed greater FA in the splenium of the corpus callosum. Boys displayed greater FA in associative white matter regions including the frontal lobes. Greater MD in boys was found in the corticospinal tract at the level of the internal capsule and below as well as in the right frontal lobe, while greater MD in girls was shown in the right arcuate fasciculus, right occipito-parietal white matter, and in the most superior aspect of the corticospinal tract in the right hemisphere. Sex–age interaction effects were also detected in the frontal lobes, and in the right arcuate fasciculus and right occipito-parietal white matter. The differences point to the importance of taking sex differences into account in developmental DTI studies.

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REFERENCES

Ahlhelm F, Hagen T, Schneider G, Dorenbeck U, Nabhan A, Reith W (2004): ADC mapping of normal human brain. Med Sci Monit 10:MT121–MT125.

Allen JS, Damasio H, Grabowski TJ, Bruss J, Zhang W (2003): Sexual dimorphism and asymmetries in the gray-white composition of the human cerebrum. Neuroimage 18:880–894.

Andreasen NC, Flaum M, Swayze V II, O'Leary DS, Alliger R, Cohen G, Ehrhardt J, Yuh WT (1993): Intelligence and brain structure in normal individuals. Am J Psychiatry 150:130–134.

Barnea-Goraly N, Menon V, Eckert M, Tamm L, Bammer R, Karchemskiy A, Dant CC, Reiss AL (2005): White matter development during childhood and adolescence: A cross-sectional diffusion tensor imaging study. Cereb Cortex 15:1848–1854.

Bartzokis G, Beckson M, Lu PH, Nuechterlein KH, Edwards N, Mintz J (2001): Age-related changes in frontal and temporal lobe volumes in men: A magnetic resonance imaging study. Arch Gen Psychiatry 58:461–465.

Blanton RE, Levitt JG, Peterson JR, Fadale D, Sporty ML, Lee M, To D, Mormino EC, Thompson PM, McCracken JT, Toga AW (2004): Gender differences in the left inferior frontal gyrus in normal children. Neuroimage 22:626–636.

Blatter DD, Bigler ED, Gale SD, Johnson SC, Anderson CV, Burnett BM, Parker N, Kurth S, Horn SD (1995): Quantitative volumetric analysis of brain MR: Normative database spanning 5 decades of life. AJNR Am J Neuroradiol 16:241–251.

Buchel C, Raedler T, Sommer M, Sach M, Weiller C, Koch MA (2004): White matter asymmetry in the human brain: A diffusion tensor MRI study. Cereb Cortex 14:945–951.

Casey BJ, Giedd JN, Thomas KM (2000): Structural and functional brain development and its relation to cognitive development. Biol Psychol 54:241–257.

Caviness VS Jr, Kennedy DN, Richelme C, Rademacher J, Filipek PA (1996): The human brain age 7–11 years: A volumetric analysis based on magnetic resonance images. Cereb Cortex 6:726–736.

Chang LC, Jones DK, Pierpaoli C (2005): RESTORE: Robust estimation of tensors by outlier rejection. Magn Reson Med 53:1088–1095.

Cohen J (1988): Statistical Power Analysis for the Behavioral Sciences, 2nd ed. Hillsdale, NJ: Lawrence Erlbaum Associates.

Courchesne E, Chisum HJ, Townsend J, Cowles A, Covington J, Egaas B, Harwood M, Hinds S, Press GA (2000): Normal brain development and aging: Quantitative analysis at in vivo MR imaging in healthy volunteers. Radiology 216:672–682.

De Bellis MD, Keshavan MS, Beers SR, Hall J, Frustaci K, Masalehdan A, Noll J, Boring AM (2001): Sex differences in brain maturation during childhood and adolescence. Cereb Cortex 11:552–557.

de Courten-Myers GM (1999): The human cerebral cortex: Gender differences in structure and function. J Neuropathol Exp Neurol 58:217–226.

Ducharme JR, Forest MG (1993): Normal pubertal development. In: Bertrand J, Rappaport R, Sizonenko PC, editors. Pediatric Endocrinology: Physiology, and Clinical Aspects, 2nd ed. Baltimore, MD: Williams and Wilkins. pp 372–386.

Duong TQ, Sehy JV, Yablonskiy DA, Snider BJ, Ackerman JJ, Neil JJ (2001): Extracellular apparent diffusion in rat brain. Magn Reson Med 45:801–810.

Faul F, Erdfelder E, Lang A-G, Buchner A: G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods(in press).

Fields DR (2005): Myelination: An overlooked mechanism of synaptic plasticity? Neuroscientist 11:528–531.

Filipek PA, Richelme C, Kennedy DN, Caviness VS Jr. (1994): The young adult human brain: An MRI-based morphometric analysis. Cereb Cortex 4:344–360.

Fountas KN, Kapsalaki EZ, Feltes CH, Smisson HF III, Johnston KW, Robinson JS Jr. (2004): Intracranial temperature: Is it different throughout the brain? Neurocrit Care 1:195–199.

Gur RC, Turetsky BI, Matsui M, Yan M, Bilker W, Hughett P, Gur RE (1999): Sex differences in brain gray and white matter in healthy young adults: Correlations with cognitive performance. J Neurosci 19:4065–4072.

Gur RC, Gunning-Dixon F, Bilker WB, Gur RE (2002): Sex differences in temporo-limbic and frontal brain volumes of healthy adults. Cereb Cortex 12:998–1003.

Haier RJ 1993: Cerebral glucose metabolism and intelligence. In:Vernon PA, editor. Biological Approaches to the Study of Human Intelligence. Norwood, NJ: Ablex. pp317–332.

Haier RJ, Jung RE, Yeo RA, Head K, Alkire MT (2005): The neuroanatomy of general intelligence: Sex matters. Neuroimage 25: 320–327.

Haut KM, Barch DM (2006): Sex influences on material-sensitive functional lateralization in working and episodic memory: Men and women are not all that different. Neuroimage 32:411–422.

Highley JR, Esiri MM, McDonald B, Cortina-Borja M, Herron BM, Crow TJ (1999): The size and fibre composition of the corpus callosum with respect to gender and schizophrenia: A post-mortem study. Brain 122 (Part 1):99–110.

Highley JR, DeLisi LE, Roberts N, Webb JA, Relja M, Razi K, Crow TJ (2003): Sex-dependent effects of schizophrenia: An MRI study of gyral folding, and cortical and white matter volume. Psychiatry Res 124:11–23.

Hiscock M, Inch R, Jacek C, Hiscock-Kalil C, Kalil KM (1994): Is there a sex difference in human laterality? I. An exhaustive survey of auditory laterality studies from six neuropsychology journals. J Clin Exp Neuropsychol 16:423–435.

Hiscock M, Israelian M, Inch R, Jacek C, Hiscock-Kalil C (1995): Is there a sex difference in human laterality? II. An exhaustive survey of visual laterality studies from six neuropsychology journals. J Clin Exp Neuropsychol 17:590–610.

Hiscock M, Perachio N, Inch R (2001): Is there a sex difference in human laterality? IV. An exhaustive survey of dual-task interference studies from six neuropsychology journals J Clin Exp Neuropsychol 23:137–148.

Huttenlocher PR, Dabholkar AS (1997): Regional differences in synaptogenesis in human cerebral cortex. J Comp Neurol 387:167–178.

Huttenlocher PR, de Courten C (1987): The development of synapses in striate cortex of man. Hum Neurobiol 6:1–9.

Jones DK, Horsfield MA, Simmons A (1999): Optimal strategies for measuring diffusion in anisotropic systems by magnetic resonance imaging. Magn Reson Med 42:515–525.

Jung RE, Haier RJ, Yeo RA, Rowland LM, Petropoulos H, Levine AS, Sibbitt WL, Brooks WM (2005): Sex differences in *N*-acetylaspartate correlates of general intelligence: An 1H-MRS study of normal human brain. Neuroimage 26:965–972.

Kim JH, Ellman A, Juraska JM (1996): A re-examination of sex differences in axon density and number in the splenium of the rat corpus callosum. Brain Res 740:47–56.

Ledberg A, Akerman S, Roland PE (1998): Estimation of the probabilities of 3D clusters in functional brain images. Neuroimage 8:113–128.

Luders E, Narr KL, Thompson PM, Woods RP, Rex DE, Jancke L, Steinmetz H, Toga AW (2005): Mapping cortical gray matter in the young adult brain: Effects of gender. Neuroimage 26:493–501.

Nopoulos P, Flaum M, O'Leary D, Andreasen NC (2000): Sexual dimorphism in the human brain: Evaluation of tissue volume, tissue composition and surface anatomy using magnetic resonance imaging. Psychiatry Res 98:1–13.

Oldfield RC (1971): The assessment and analysis of handedness: The Edinburgh inventory. Neuropsychologia 9:97–113.

Passe TJ, Rajagopalan P, Tupler LA, Byrum CE, MacFall JR, Krishnan KR (1997): Age and sex effects on brain morphology. Prog Neuropsychopharmacol Biol Psychiatry 21:1231–1237.

Pfefferbaum A, Rosenbloom M, Deshmukh A, Sullivan E (2001): Sex differences in the effects of alcohol on brain structure. Am J Psychiatry 158:188–197.

Pfleiderer B, Ohrmann P, Suslow T, Wolgast M, Gerlach AL, Heindel W, Michael N (2004): *N*-acetylaspartate levels of left frontal cortex are associated with verbal intelligence in women but not in men: A proton magnetic resonance spectroscopy study. Neuroscience 123:1053–1058.

Plante E, Schmithorst VJ, Holland SK, Byars AW (2006): Sex Differences in the activation of language cortex during childhood. Neuropsychologia 44:1210–1221.

Pujol J, Soriano-Mas C, Ortiz H, Sebastian-Galles N, Losilla JM, Deus J (2006): Myelination of language-related areas in the developing brain. Neurology 66:339–343.

Reiss AL, Abrams MT, Singer HS, Ross JL, Denckla MB (1996): Brain development, gender and IQ in children. A volumetric imaging study. Brain 119 (Part 5):1763–1774.

Rivkin MJ (2000): Developmental neuroimaging of children using magnetic resonance techniques. Ment Retard Dev Disabil Res Rev 6:68–80.

Ruff RM, Parker SB (1993): Gender- and age-specific changes in motor speed and eye-hand coordination in adults: Normative values for the Finger Tapping and Grooved Pegboard Tests. Percept Mot Skills 76:1219–1230.

Schlaggar BL, Brown TT, Lugar HM, Visscher KM, Miezin FM, Petersen SE (2002): Functional neuroanatomical differences between adults and school-age children in the processing of single words. Science 296:1476–1479.

Schmithorst VJ, Dardzinski BJ (2002): Automatic gradient preemphasis adjustment: a 15-minute journey to improved diffusion-weighted echo-planar imaging. Magn Reson Med 47:208–212.

Schmithorst VJ, Holland SK (2006): Functional MRI evidence for disparate developmental processes underlying intelligence in boys and girls. Neuroimage 31:1366–1379.

Schmithorst VJ, Holland SK (2007): Sex differences in the development of neuroanatomical functional connectivity underlying intelligence found using bayesian connectivity analysis. Neuroimage 35:406–419.

Schmithorst VJ, Dardzinski BJ, Holland SK (2001): Simultaneous correction of ghost and geometric distortion artifacts in EPI using a multiecho reference scan. IEEE Trans Med Imaging 20:535–539.

Schmithorst VJ, Wilke M, Dardzinski BJ, Holland SK (2002): Correlation of white matter diffusivity and anisotropy with age during childhood and adolescence: A cross-sectional diffusion-tensor MR imaging study. Radiology 222:212–218.

Schmithorst VJ, Wilke M, Dardzinski BJ, Holland SK (2005): Cognitive functions correlate with white matter architecture in a normal pediatric population: A diffusion tensor MRI study. Hum Brain Mapp 26:139–147.

Shaywitz BA, Shaywitz SE, Pugh KR, Constable RT, Skudlarski P, Fulbright RK, Bronen RA, Fletcher JM, Shankweiler DP, Katz L, Gore JC (1995): Sex differences in the functional organization of the brain for language. Nature 373:607–609.

Szaflarski JP, Holland SK, Schmithorst VJ, Byars AW (2006): fMRI study of language lateralization in children and adults. Hum Brain Mapp 27:202–212.

Szeszko PR, Vogel J, Ashtari M, Malhotra AK, Bates J, Kane JM, Bilder RM, Frevert T, Lim K (2003): Sex differences in frontal lobe white matter microstructure: A DTI study. Neuroreport 14:2469–2473.

Westerhausen R, Walter C, Kreuder F, Wittling RA, Schweiger E, Wittling W (2003): The influence of handedness and gender on the microstructure of the human corpus callosum: A diffusion-tensor magnetic resonance imaging study. Neurosci Lett 351:99–102.

Westerhausen R, Kreuder F, Dos Santos Sequeira S, Walter C, Woerner W, Wittling RA, Schweiger E, Wittling W (2004): Effects of handedness and gender on macro- and microstructure of the corpus callosum and its subregions: A combined high-resolution and diffusion-tensor MRI study. Brain Res Cogn Brain Res 21:418–426.

Wilke M, Schmithorst VJ, Holland SK (2002): Assessment of spatial normalization of whole-brain magnetic resonance images in children. Hum Brain Mapp 17:48–60.

Wilke M, Schmithorst VJ, Holland SK (2003a): Normative pediatric brain data for spatial normalization and segmentation differs from standard adult data. Magn Reson Med 50:749–757.

Wilke M, Sohn JH, Byars AW, Holland SK (2003b): Bright spots: Correlations of gray matter volume with IQ in a normal pediatric population. Neuroimage 20:202–215.

Zhou M, Goto N, Goto J, Moriyama H, He HJ (2000): Gender dimorphism of axons in the human lateral corticospinal tract. Okajimas Folia Anat Jpn 77:21–27.